PATENT

Case Docket No.: NOVCEL.3CPDDDVC
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Applicant(s)	:	**Hubbell, et al.**
App. No.	:	**10/761,180**
Filed	:	**January 20, 2004**
For	:	**Gels For Encapsulation Of Biological Materials**
Examiner	:	**Berman, Susan W.**
Group Art Unit	:	**1711**

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on

March 1, 2006
(Date)

Kirk Hahn, Reg. No. 51,763

DECLARATION UNDER 37 C.F.R. §1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, AMARPREET S. SAWHNEY, a citizen of the United States, hereby declare that:

2. My address is 101A First Ave., Waltham, MA 02451.

3. I am a co-inventor of the invention in U.S. Application Serial No. **10/761,180** entitled **"Gels For Encapsulation Of Biological Materials"** (the "Application"); and U.S. Application Serial No. 07/843,485 ("Priority Document I"), now abandoned, filed February 28, 1992 and U.S. Application Serial No. 07/870,540 ("Priority Document II"), filed April 20, 1992, now abandoned, on which this application claims priority.

4. I understand that the Examiner has cited Soon-Shiong et al., U.S. Patent Numbers 5,700,848; 5,705,270 and 5,846,530 in her rejection of the claims presented in the Application. I also understand that Soon-Shiong '848, '270 and '530 have effective filing dates of October 29, 1991.

5. Prior to October 29, 1991; I reduced to practice in the United States a method of encapsulating biological materials, which comprises mixing the biological material with a polymer, forming microcapsules, coating the microcapsules with a photoinitiator, mixing the microcapsules containing the biological material in an aqueous macromer, polymerizing the gel using a light source, and generating macrocapsules in accordance with the subject matter claimed

in this application.

6. Attached hereto is a copy of a page from my laboratory notebook ("Exhibit 1"), which shows a protocol for encapsulating cells in alginate-PLL microcapsules. Except for the masked date, Exhibit 1 is a true and correct copy of the relevant page in the original bound laboratory notebook.

7. Attached hereto is a copy of a page from my laboratory notebook ("Exhibit 2") bearing a date before October 29, 1991. Except for the masked date, Exhibit 2 is a true and correct copy of the relevant page in the original bound laboratory notebook. Exhibit 3 is a transcription of the handwriting of Exhibit 2.

8. Exhibit 2 shows the protocol for encapsulating alginate-PLL microcapsules in a photopolymerized PEO gel. The steps in the protocol correspond to the steps in the application.

9. Accordingly, Exhibit 1 and Exhibit 2 demonstrate that the invention as described in the Application was conceived and reduced to practice in the United States before the filing date of Soon-Shiong '848.

10. I understand that the Examiner has also cited Soon-Shiong et al., U.S. Patent Numbers 5,545,423; 5,759,578; 5,788,988 and 5,879,709 in her rejection of the claims presented in the Application. I understand that Soon-Shiong '423, '578, '988, and '709 have effective filing dates of November 25, 1991, which is later than Soon-Shiong '848. Therefore, as shown above, the invention was conceived and reduced to practice in the United States before the effective filing date of Soon-Shiong '423.

11. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. Moreover, these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

2/22/06
Date: _____

_____
Amarpreet S. Sawhney

EXHIBIT 1

Final step

Procedure for Microencapsulation

A syringe pump with a 10-ml syringe connected to a special air jet is used for making sodium alginate islet droplets.

For each preparation, 2000–3000 islets in 0.2 ml of saline is mixed gently with 2 ml of 1.5% sodium alginate, transferred to the 10-ml syringe, and connected to the air jet. The distance from the tip of the air jet needle to the surface of the collecting fluid is set at precisely 8 cm. The syringe pump and air flow are then turned on to extrude sodium alginate droplets containing islets into 50 ml of the 1.1% $CaCl_2$ solution in a beaker. During extrusion, the islets are kept in suspension by gently rotating a small magnet inside the 10-ml syringe. After the extrusion process is completed, the spherical calcium alginate gel droplets are transferred to a 50-ml polystyrene test tube with a conical bottom, and allowed to settle before withdrawing the supernatant down to 5 ml using a vacuum aspirator. The gel droplets are washed once with 30 ml of 0.55% $CaCl_2$, once with 0.2% $CaCl_2$, and then suspended in 25 ml of 0.1% CHES solution for 3 min. After aspirating the CHES solution, the capsules are washed with 1.1% $CaCl_2$ and suspended in 25 ml of 0.05% (W/V) poly(L-lysine) for 6 min. After further washing with CHES, $CaCl_2$, and saline, the microcapsules are incubated in 0.15% sodium alginate for 4 min and washed with saline. The capsules are then suspended in 10 ml of 55 m*M* sodium citrate solution for 5 min. The final product is washed twice with saline and once with medium CMRL-1969, and then transferred to Falcon flasks for incubation at 37° until required for *in vitro* and *in vivo* studies.

distance should be ← 8 cm

4: CHES, 5: SALINE

1400 mL | mins on test tube on wall (air-pressure)

EXHIBIT 2

[redacted] PROTOCOL FOR PLL-MICROSPHERE COATING W/- PEG CELLS

Make dyn of PLL microspheres & suspend them in a saturated (possibly make) solution of ethyl eosin in a HEPES buffered saline. Aspirate off all excess $CaCl_2$ soln from spheres & add 10 ml of dye soln. Incubate for 5 min. Rinse off repeatedly (about 4-5 times) w/- 1.1% $CaCl_2$.

Aliquot 0.5 ml spheres into 15 ml centrifuge tubes. Aspirate off all excess fluid. Add 100 µl of TEA soln ( ) and add 2 ml of 30% PEO acrylate soln's. Vortex & expose to laser (514 nm) at ~100 mW for ~1-2 min with ~~constant~~ intermittent stirring. Add 10 ml saline to wash off excess polymer, aspirate & resuspend w/ extracellular soln to allow spheres to re-swell. Wash twice w/ extracellular & then w/- saline. Re-suspend in 5 ml saline & transplant in 2 mice.

EXHIBIT 3

EXHIBIT 3

Transcription of handwriting of Exhibit 2

PROTOCOL FOR PLL-MICROSPHERE COATING W/ PEO GELS

Make algin -/PLL microspheres & immerse them in a saturated (freshly made) solution of ethyl eosin in HEPES buffered saline. Aspirate of[1] all excess $CaCl_2$ sol^[2] from spheres (5 ml) and add 10 ml of dye sol^. Incubate for 5 min. Rinse off repeatedly (about 4 – 5 times) w/- 1.1% $CaCl_2$.

Aliquot 0.5 ml spheres into 15 ml centrifuge tubes. Aspirate off all excess fluid. Add 10 µl of TEA[3] sol^ ( ) and add 2 ml of 30% PEO[4] acrylate sol^'s. Vortex & expose to laser (514 nm) at ~100 mW for ~1-2 min with intermediate stirring. Add 10 ml saline to wash off excess polymer, aspirate & incubate w/- citrate sol^ to allow spheres to re-swell. Wash twice w/- citrate and then w/- saline. Suspend in 5 ml saline & implant in 2 mice.

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[1] "of" is off

[2] "sol^" is solution

[3] "TEA" is triethanol amine

[4] "PEO" is poly(ethylene oxide)